

SUPPORTING INFORMATION

for

Melting of a β -hairpin peptide using isotope-edited 2D IR spectroscopy and simulations

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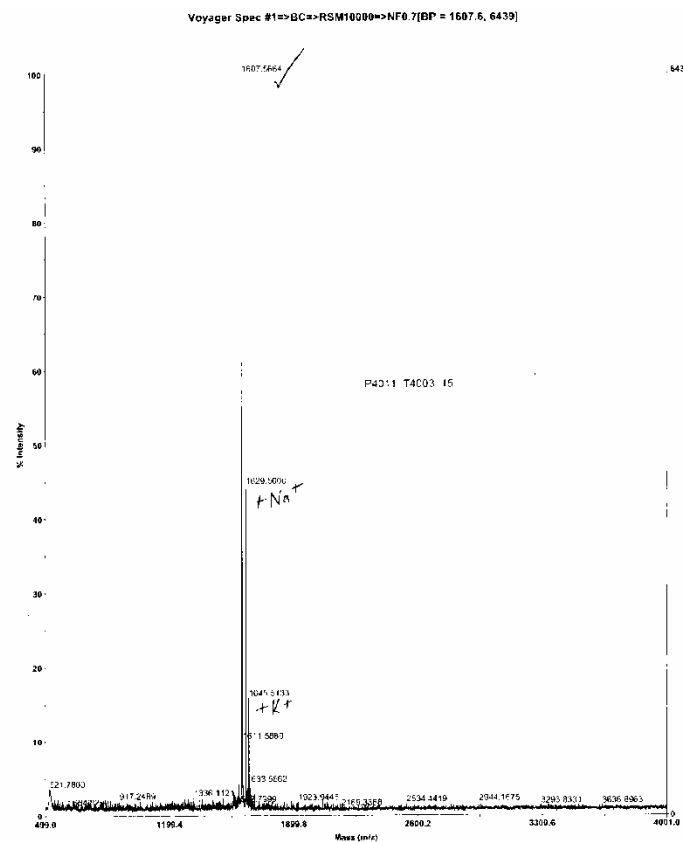
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Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

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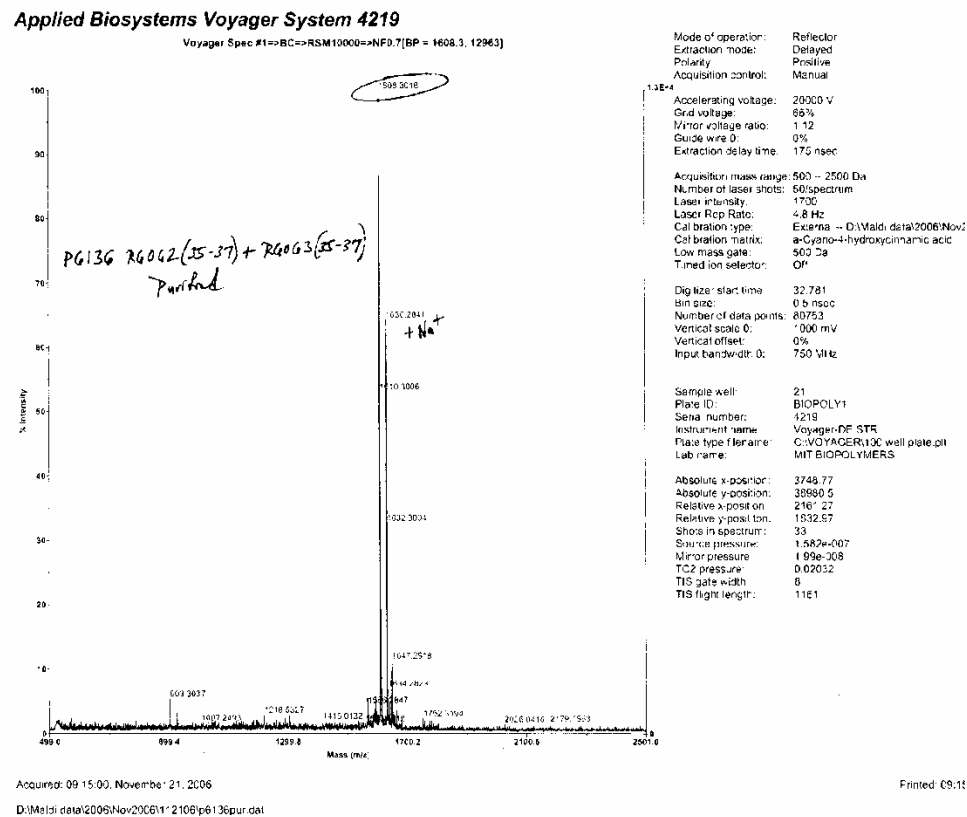
1. Mass spectra of the synthesized peptides

1.a. TZ2-UL mass spectrum recorded on an Applied Biosystems Voyager MALDI-TOF mass spectrometer on February 3, 2004. The expected mass of TZ2-UL is 1608 g/mol, and the recorded mass is 1607.6 g/mol.



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Acquired: '3 54 00, February 05, 2004

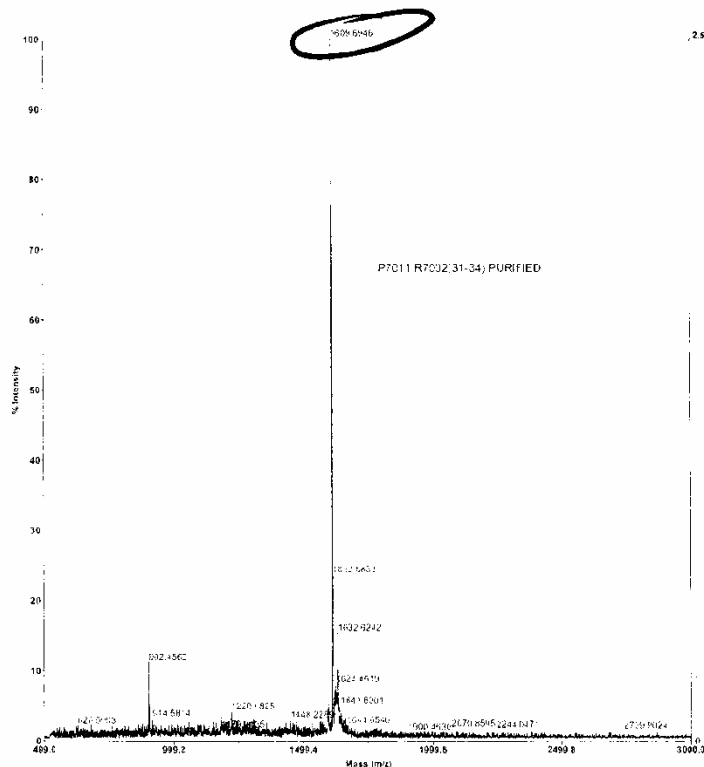
1.b. TZ2-K8 mass spectrum recorded on an Applied Biosystems Voyager MALDI-TOF mass spectrometer on November 21, 2006. The expected mass of TZ2-K8(¹³C) is 1609 g/mol. The recorded mass is 1608.3 g/mol, which is approximately 1 a.m.u. higher than the recorded mass of TZ2-UL (Fig. 2.a.1).



1.c. TZ2-S1 mass spectrum recorded on an Applied Biosystems Voyager MALDI-TOF mass spectrometer. The expected mass of TZ2-S1(¹⁸O) is 1610 g/mol. The recorded mass is 1609.6 g/mol, which is approximately 2 a.m.u. higher than the recorded mass of TZ2-UL.

Applied Biosystems Voyager System 4219

Voyager Spec #1=>BC=>RSM10000=>NF0 7[RP = 1609 7, 25228]



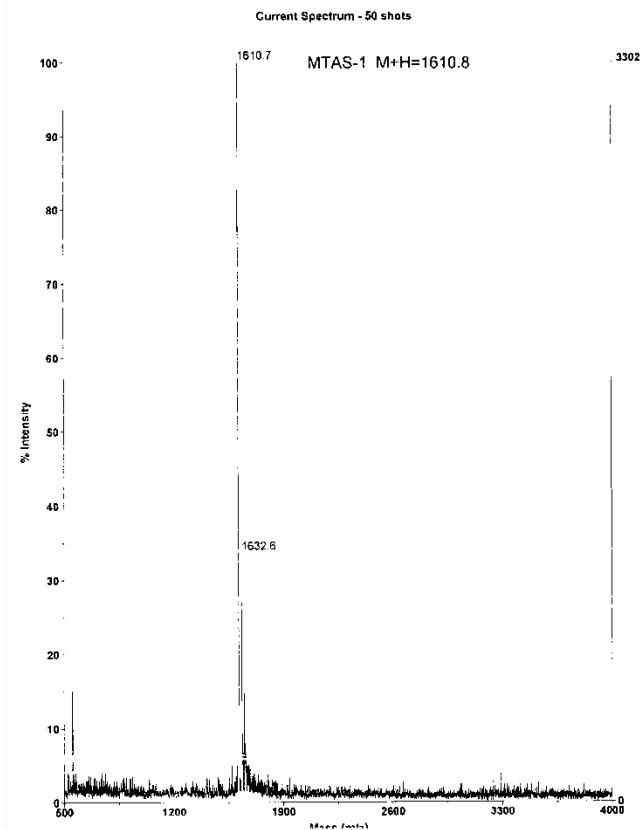
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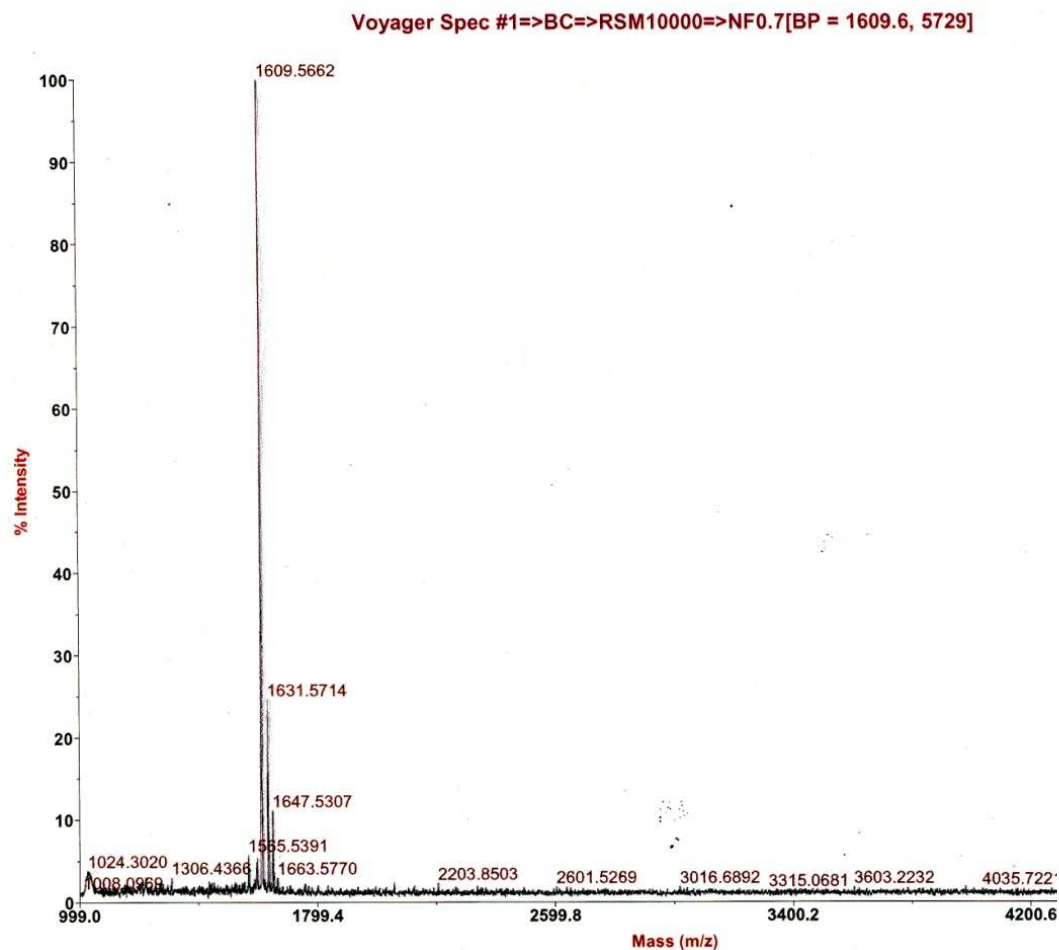
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Extraction mode: Delayed
Polarity: Positive
Acquisition control: Manual
Accelerating voltage: 20000 V
Grid voltage: 60V
Mirror voltage ratio: 1:12
Guide wire 0: 0V
Extraction delay time: 175 nsec
Acquisition mass range: 500 -- 5000 Da
Number of laser shots: 50/spectrum
Laser intensity: 1904
Laser Rep Rate: 2.7 Hz
Calibration type: External -- D:\Maldi data\2007\Feb2007\0221C7\essing.dat
Calibration matrix: a-Cyano-4-hydroxycinnamic acid
Low mass gate: 500 Da
Timed ion selector: Off
Digitizer start time: 32.762
Bin size: 0.5 nsec
Number of data points: 141253
Vertical scale 0: 1000 mV
Vertical offset: 0V
Input bandwidth 0: 750 MHz
Sample well: 97
Plate ID: BIOPOLY2
Serial number: 4219
Instrument name: Voyager-DE STR
Plate type filename: C:\VOYAGER\100 well plate.plt
Lab name: MIT BIOPOLYMERS
Absolute x-position: 32312.1
Absolute y-position: 1145.26
Relative x-position: 244.628
Relative y-position: -441.237
Shots in spectrum: 12
Source pressure: 9.1e-006
Mirror pressure: 1.67e-006
TOF pressure: 0.02164
TIS gate width: 8
TIS flight length: 1161

Printed: 11:1

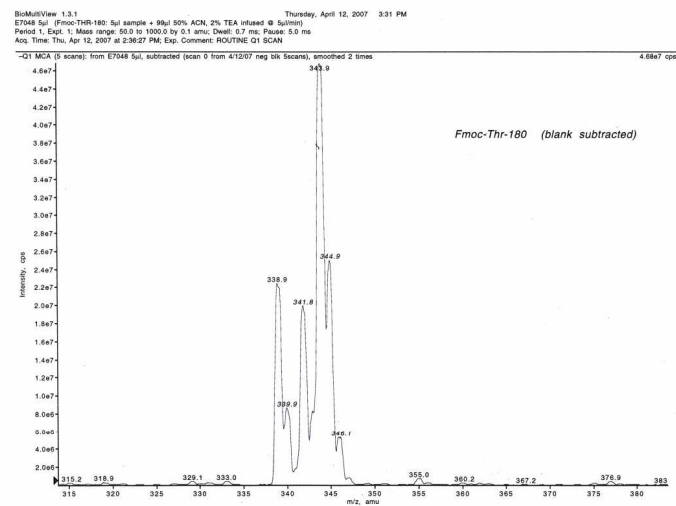
1.d. TZ2-TT mass spectrum provided by Anaspec Inc. The expected mass of TZ2-T3(¹³C)T10(¹³C) is 1610 g/mol, which agrees with the recorded mass of 1610.7.



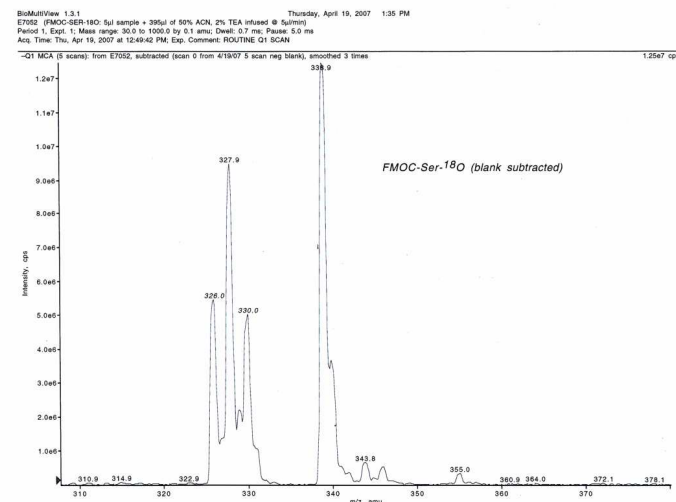
1.e. TZ2-T10 mass spectrum recorded on an Applied Biosystems Voyager MALDI-TOF mass spectrometer. The expected mass of TZ2-T10(^{18}O) is 1610 g/mol. The recorded mass is 1609.6 g/mol, which is approximately 2 a.m.u. higher than the recorded mass of TZ2-UL.



D:\...T8026(36_41) TZ2.dat
Acquired: 18:33:00, October 21, 2008



1.f. ^{18}O labeled Fmoc-Thr-OH mass spectrum recorded on a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS). The expected mass of unlabeled Fmoc-Thr-OH is 341.4 g/mol. The recorded masses are 341.8, 343.9, 344.9, and 346.1 g/mol. These masses represent a distribution in isotope label incorporation into Fmoc-Thr-OH.

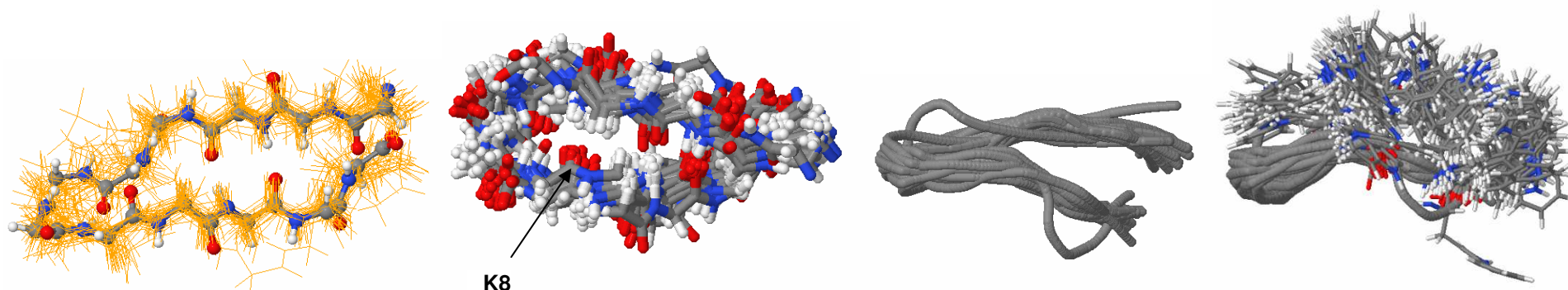


1.g. ^{18}O labeled Fmoc-Ser-OH mass spectrum recorded on a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS). The expected mass of unlabeled Fmoc-Ser-OH is 327.3 g/mol. The recorded masses are 326.0, 327.9, and 330.0 g/mol. These masses represent a distribution in isotope label incorporation into Fmoc-Ser-OH.

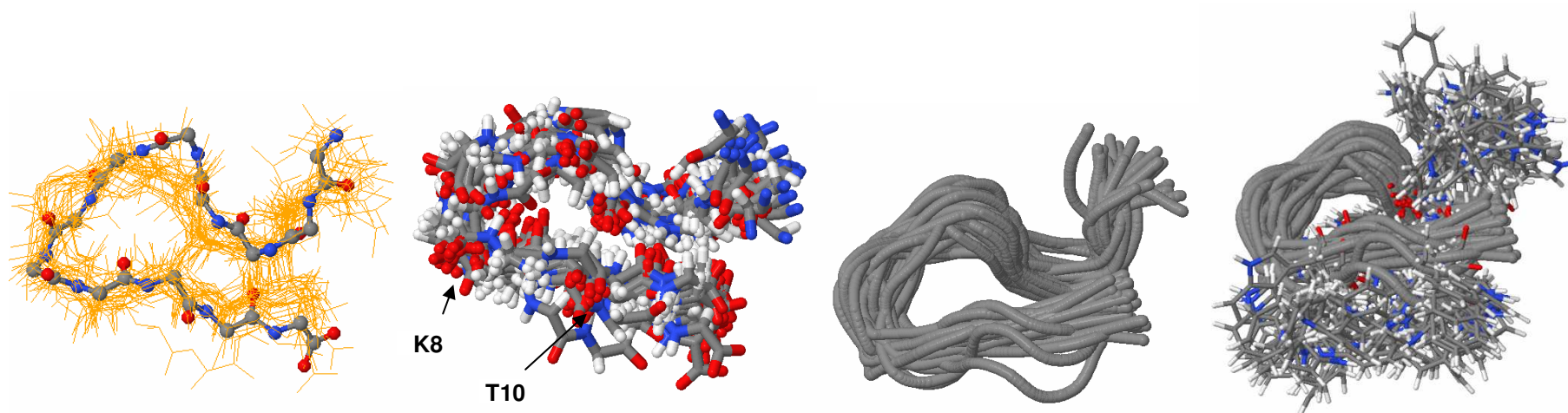
2. Visualization of the Markov States: Backbone conformation and tryptophan packing

Here we provide different visualizations of TZ2 for the 30 initial structures within each simulated Markov state. Backbone visualizations serve to characterize the amide group orientations and conformational disorder of each state, and the right panel illustrates the packing of tryptophans for each state. Structures are oriented in a manner that coincides roughly with Fig. 9.

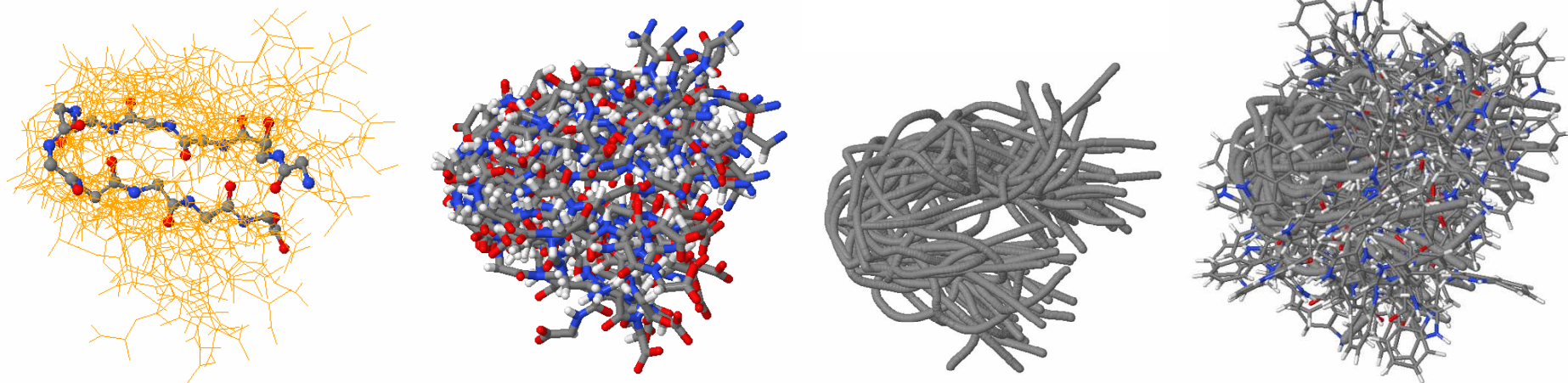
“Folded” (**FO**, 250851)



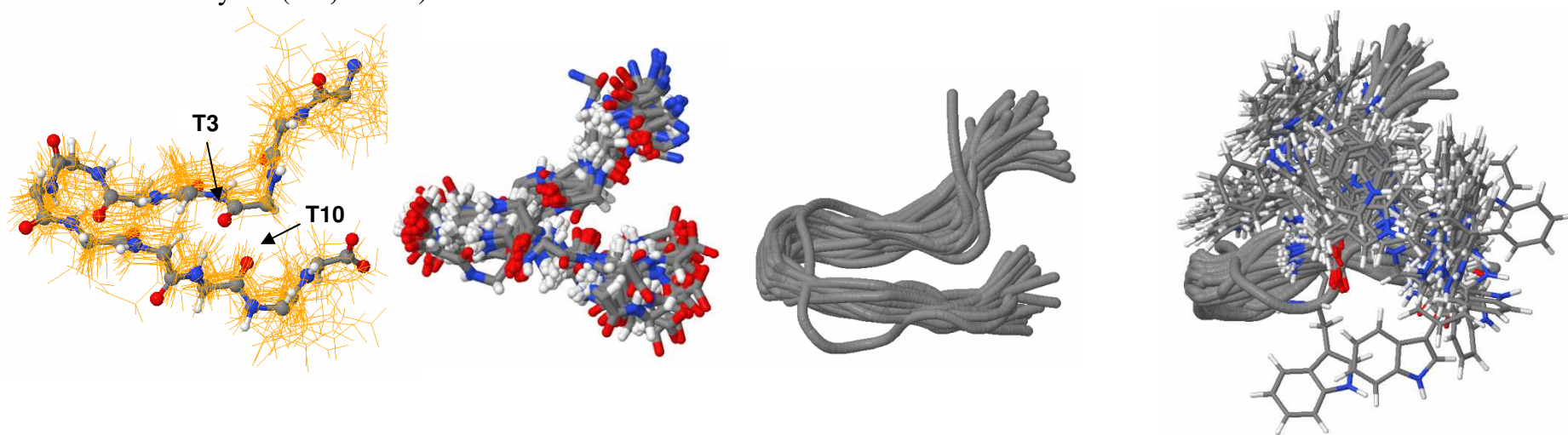
“Bulged” (**BT**, 214369)



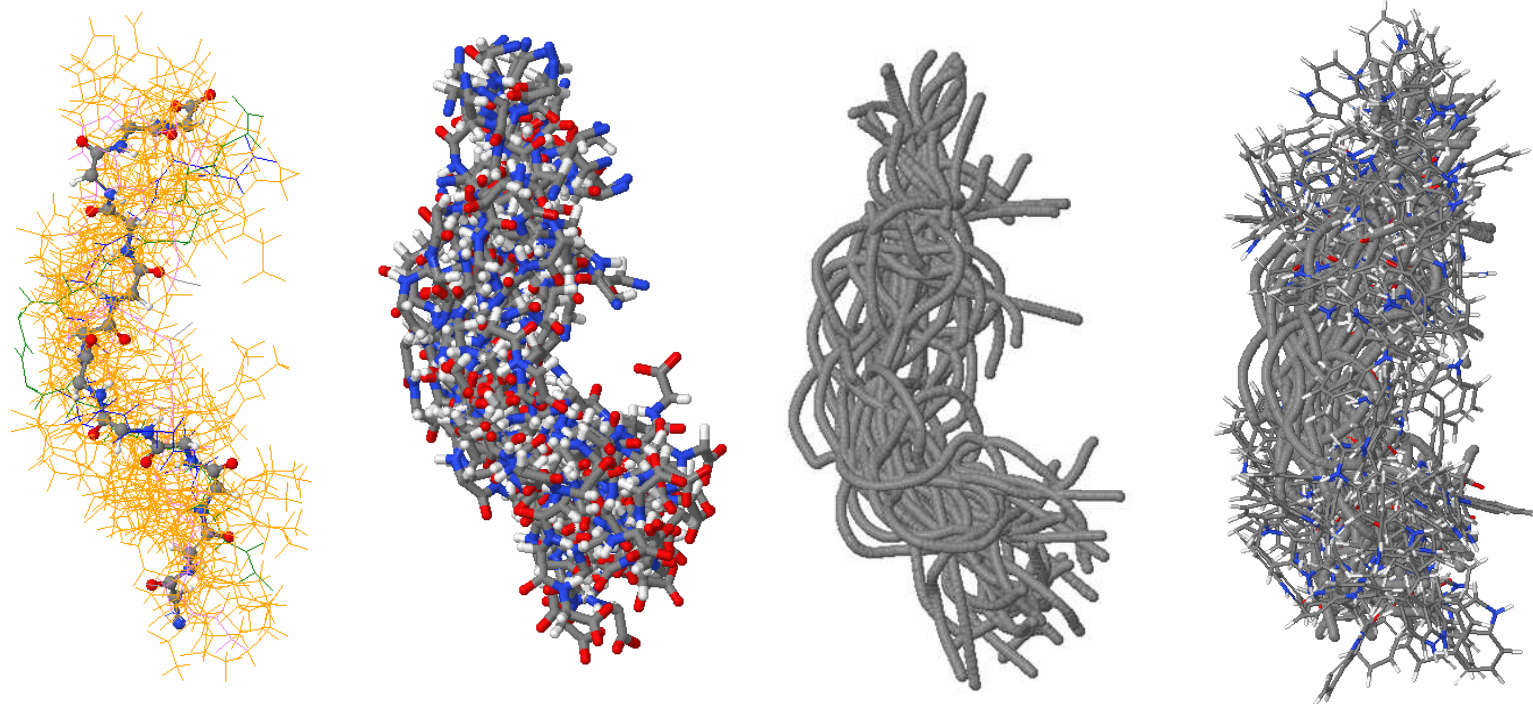
“Disordered” (SR, 271154)



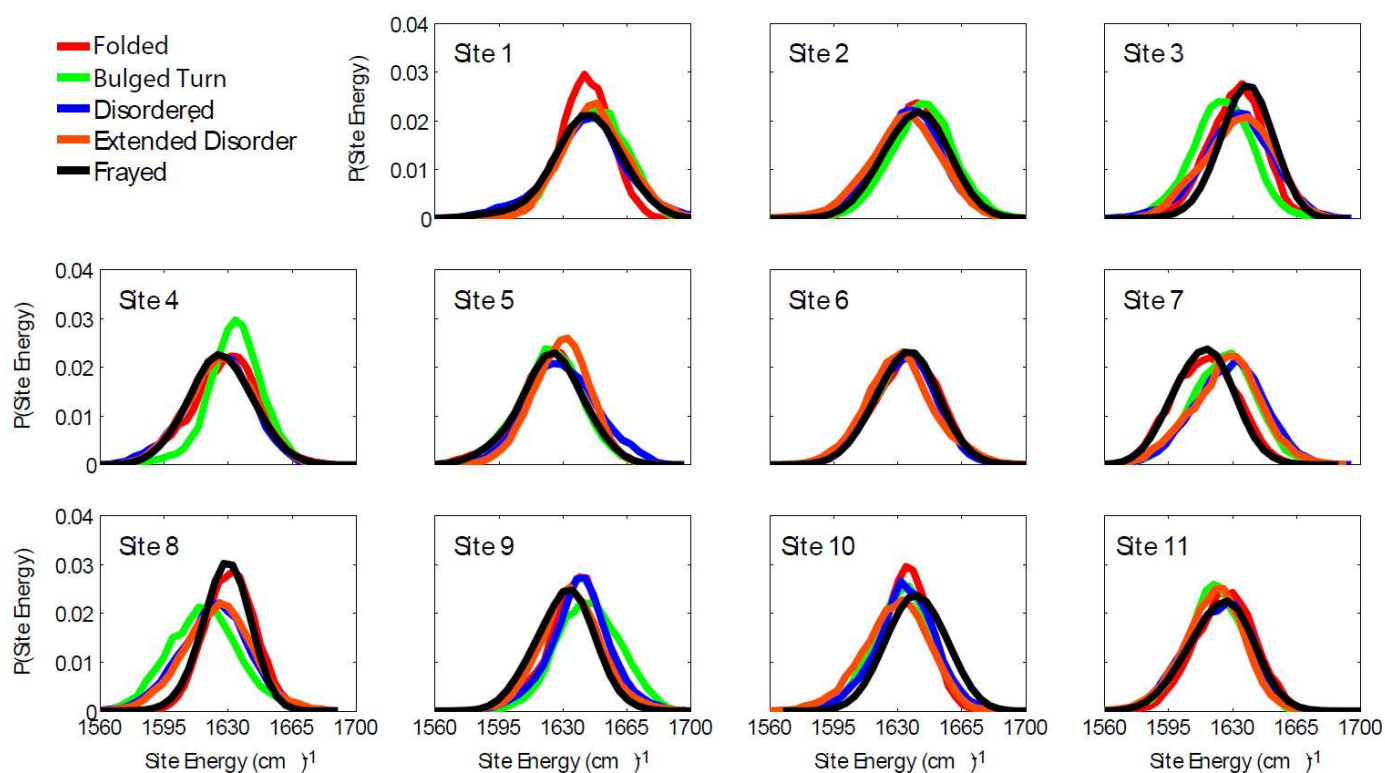
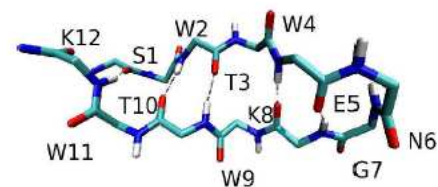
“Frayed” (FR, 11131)



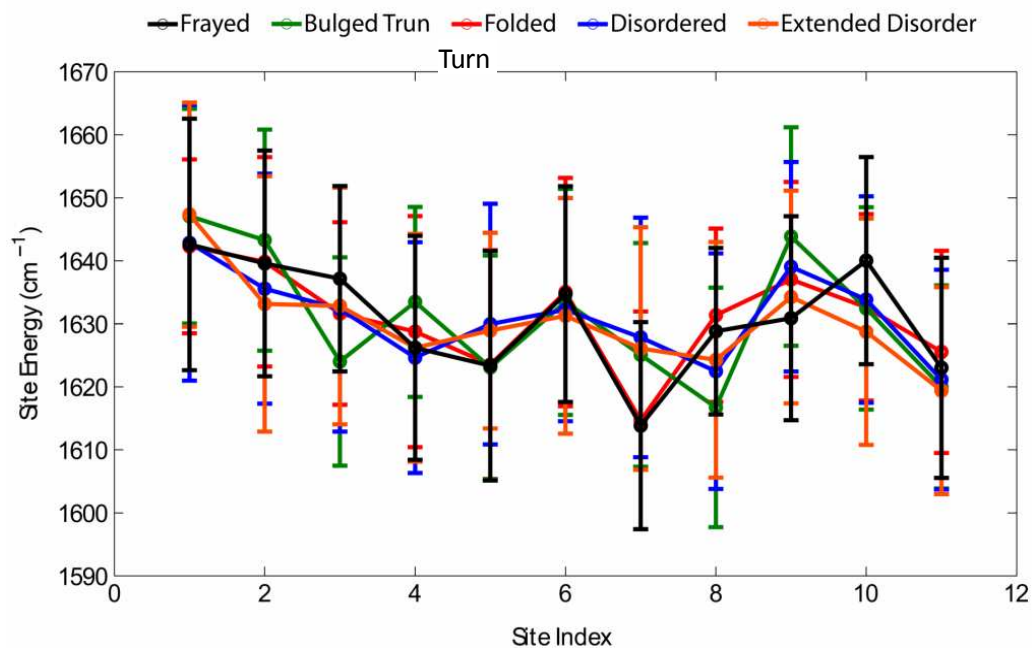
“Extended Disorder” (ED, 64336)



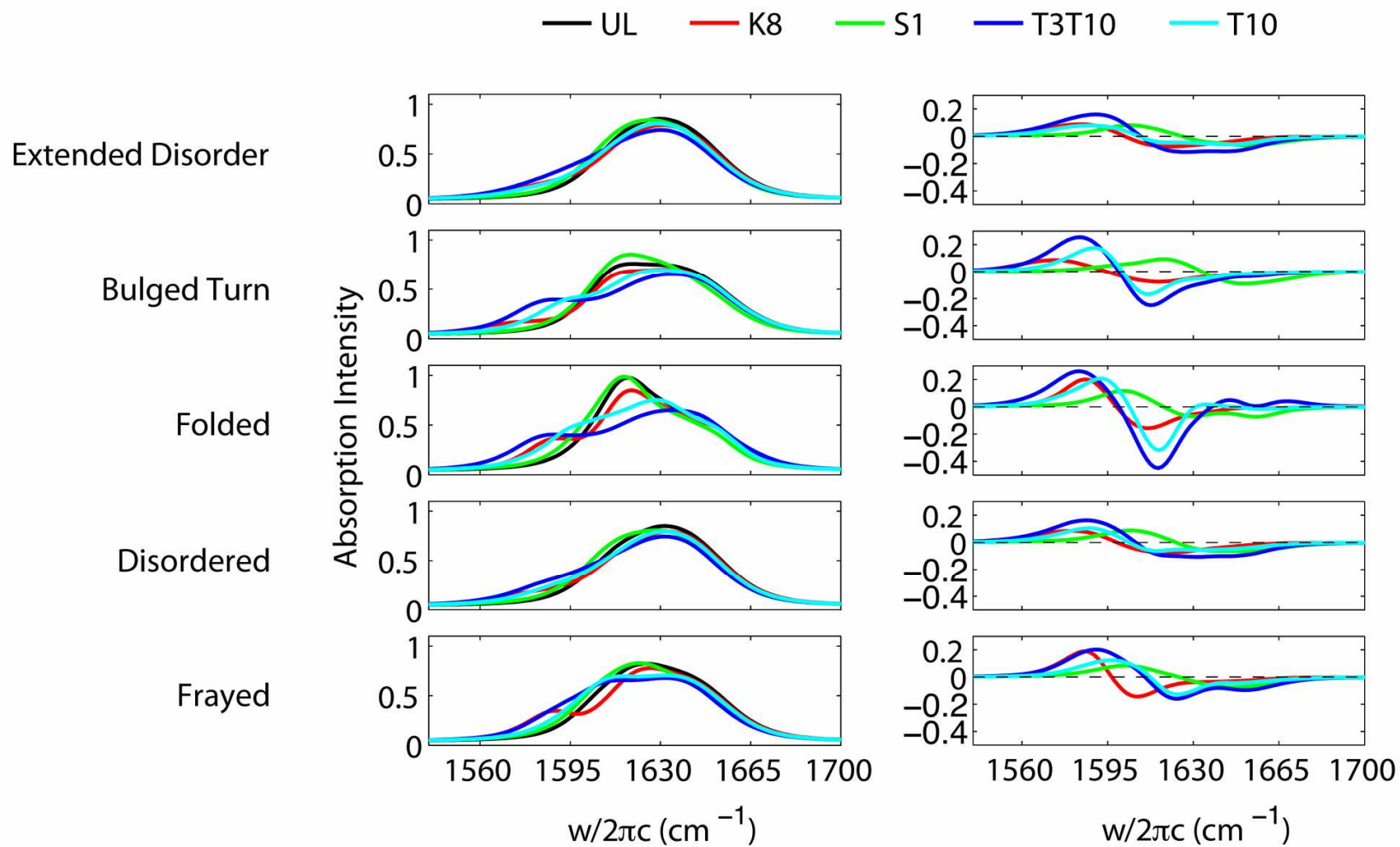
3. Analysis of simulated site energy variation by conformer



Site energy mean values showing error bars with one standard deviation



4. Simulated FTIR and FTIR difference spectra for isotopologues of all Markov States

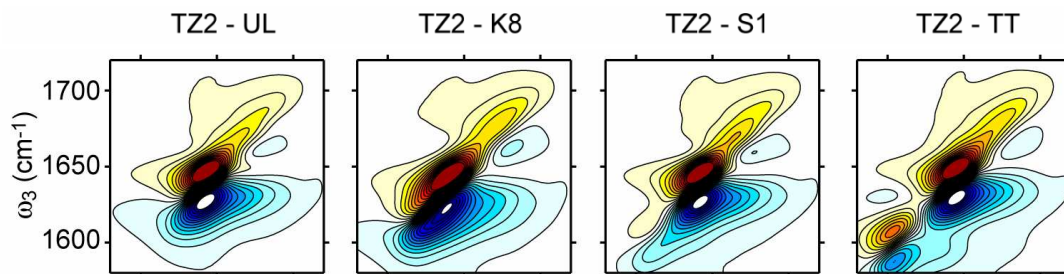


5. Comparison of spectral features in simulations with experiment.

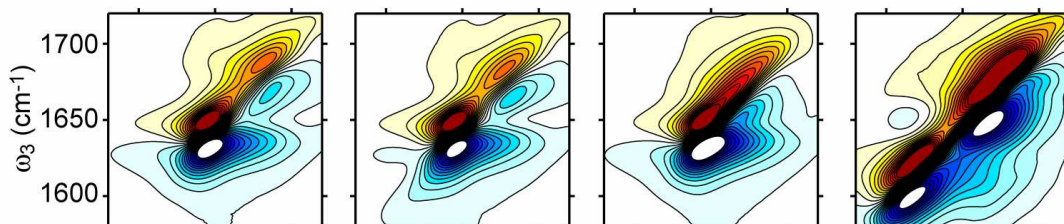
All units in cm^{-1} .

Experiment (25°C)						Experiment (75°C)					Simulations																	
Site	Absorbance Maximum			FWHM^		Absorbance Maximum			FWHM		64336 Frayed (FR)			214369 Bulged (BT)			250851 Folded (FO)			271154 Disordered (SR)								
	Peak		Width	Peak		Width	Peak		Width	Peak		Width	Peak		Width	Peak		Width	Peak		Width							
	Ex*	Iso**	Iso2	Iso	Iso2	Ex*	Iso**	Iso2	Iso	Iso2	Ex	Iso	Iso	Ex	Iso	Iso	Ex	Iso	Iso	Ex	Iso	Iso						
UL	1636					1637					1649				1645				1651				1646					
S1	1633	1629		10		1635					1647	1637	4.6		1660	1647	6.5		1651	1635	5.7		1646	1639	6.8			
K8	1635	1598	1615	22	10	1639	1601	1617	17	10	1652	1623	13.7		1644	1608	8.3		1651	1622	5.7		1645	1616	10.8			
T 10	1639	1628		9		1644	1632		9		1649	1624	9.8		1653	1628	6.3		1640	1627	3.3		1649	1624	8.3			
TT	1635	1603		19		1634	1602		17		1651	1629	13.1		1629	1616	6.6		1645	1622	6.0		1647	1622	10.9			
* Excitonic ¹² C band absorbance maximum																												
** Isotope shifted peak absorbance maximum																												
^ Diagonal peak width (FWHM)																												

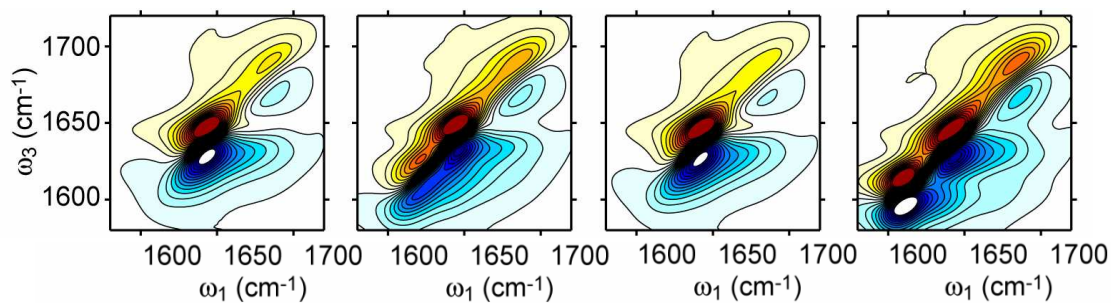
6. 2D IR spectral simulations of TZ2 isotopologues for specific conformations



NL simulation for single structure with intact S1-W12 hydrogen bond.



Slipped registry simulation for a single +1C slipped registry conformer with 4 hydrogen bonds.



Simulations of the 2D IR spectra for the NMR structure of TZ2.